ORIGINAL ARTICLE

Regular tai chi chuan exercise enhances functional mobility and CD4CD25 regulatory T cells

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Background: The duration and vigour of physical exercise are widely considered to be critical elements that may positively or negatively affect physical health and immune response.
Objectives: To investigate the effect of a 12 week programme of regular tai chi chuan exercise (TCC) on functional mobility, beliefs about benefits of exercise on physical and psychological health, and immune regulation in middle aged volunteers.
Methods: This quasi-experimental research design involving one group with testing before and after the programme was conducted to measure the effect of 12 weeks of TCC exercise in 14 men and 23 women from the normal community.
Results: Regular TCC exercise had a highly significant positive effect on functional mobility ($p = 0.001$) and beliefs about the health benefits of exercise ($p = 0.013$) in the 37 participants. Total white blood cell and red blood cell count did not change significantly, but a highly significant ($p<0.001$) decrease in monocyte count occurred. A significant ($p = 0.05$) increase in the ratio of T helper to suppressor cells (CD4:CD8) was found, along with a significant ($p = 0.015$) increase in CD4CD25 regulatory T cells. Production of the regulatory T cell mediators transforming growth factor β and interleukin 10 under specific antigen stimulation (varicella zoster virus) was also significantly increased after this exercise programme.
Conclusions: A 12 week programme of regular TCC exercise enhances functional mobility, personal health expectations, and regulatory T cell function.

Vigorous physical activity is usually associated with fewer days of sickness and increased risk of upper respiratory tract infections.[1 2] This has been attributed to an alteration in lymphocyte cytokine control.[2 3] Both innate and adaptive immune systems exhibit adverse changes after heavy exertion lasting longer than 90 minutes. These immune changes persist for 3–72 hours depending on the immune parameters measured.[2 4] Recent studies have also shown significantly increased concentrations of interleukin (IL) 4 in both plasma and urine,[4] suggesting increased response of T helper 2 cells. Exercise immunology studies focus almost exclusively on the early phase of vigorous exercise.[1 3–5] They identify acute insults to the immune response after single sessions of vigorous exercise. Few studies, however, observe longer term effects of athletic training. A study of elite swimmers found that cell mediated immunity remained unchanged after five months of swimming training.[6] Another study that assessed the effects of regular exercise on the lymphocyte responses of the psychological speech stressor and physical exercise showed that reduced immune reaction was found in psychological speech stressor but not physical exercise.[7] These studies show that, although vigorous exercise compromises cell immunity in the early hours to days, it does not significantly affect immunity in the long run. In fact, the volume of exercise has been shown to be a critical element in inducing a positive or negative effect on immune response.[8] It has been shown that moderate exercise enhances T cell function[8] and decreases respiratory infections.[9] Although many components of the immune system are known to exhibit various changes depending on the stage and type of exercise, the long term effects of regular moderate exercise remain unknown.

Tai chi chuan (TCC), a traditional martial art practiced in China for centuries, combines deep diaphragmatic breathing and relaxation with many fundamental postures that flow smoothly from one to the other through slow, gentle, graceful movements.[8] Physiologically, it uses less than 55% of maximum capacity.[10] TCC has been shown to have a beneficial effect on proprioception at the ankle and knee joints,[11] balance, cardiovascular and respiratory function, flexibility, muscular strength, and psychological state.[10 12 13] It has been reported to have a lower incidence of injury than tae kwon do, aikido, kung fu, and karate.[14]

Despite the widespread and growing participation in TCC, few studies have been performed on immune enhancement from TCC exercise and its biological mechanism.[10] Earlier studies on immune enhancement from TCC exercise performed at an acute stage showed increased peripheral blood activated T cells and B cells reflected by increases in E-rosette and ZC-rosette lymphocytes immediately after exercise.[15 16] Later, a study on an elderly group showed that regular TCC exercise for 15 weeks increased varicella zoster virus specific lymphocytes.[17] However, to date there is no clear information on the effect of regular TCC exercise on the function of regulatory T cells, which play an important role in mucosal defence and inflammatory disease.[18–20] In this study, we investigated the effect of regular TCC exercise on functional mobility, health expectations of exercise, and regulatory T cell function in middle aged adults.

METHODS

Subjects and study design

A quasi-experimental research design was used of one group tested at the beginning and end of a 12 week TCC exercise programme. Recruitment posters were posted in five community culture centres asking for volunteers to participate in a study of the effect of TCC exercise on physical health and

Abbreviations: IL, interleukin; OEE, outcome expectations for exercise; TCC, tai chi chuan; TGF, transforming growth factor

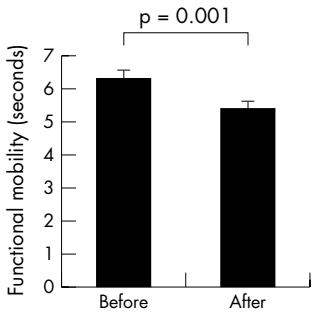

Figure 1 Changes in functional mobility as demonstrated by the "get up and go" test before and after the 12 week tai chi chuan exercise.

immunity in Kaohsiung County, Taiwan. Eligibility was determined from geographical accessibility, ability to commit to the research programme and time schedule, and medical background. Subjects with cardiopulmonary illness, autoimmune disorder, malignancies actively being treated with chemotherapy, or diseases under corticosteroid treatment were excluded. There were 40 participants at the beginning of the study, and 37 at the end. Three dropped out because of irregular participation. There were 14 male (38%) and 23 female (62%) participants with a mean (SE) age of 55.41 (1.77). Informed consent was obtained from each participant to commit to the exercise schedule, to complete questionnaires before and after each exercise session, and have immune blood tests at the beginning and end of the programme. The study protocol was approved by the Kaohsiung Chang Gung Memorial Hospital Review Board.

TCC exercise programme

TCC exercise is referred to as "meditation through movement," incorporating elements of balance, postural alignment, and concentration.[7] Subjects learned to perform Cheng's 37 standardised movements (tai chi 37 forms),[21] under the guidance of a TCC master with 30 years of experience, who led all exercise sessions throughout the 12 week intervention period. Each participant also received a take home video tape demonstrating the tai chi 37 forms before the programme. Each TCC session lasted 60 minutes with a 10 minute warm up (including stretching and balancing), a 40 minute practice, and a 10 minute cool down. Group sessions were given three times a week from 0800 to 0900 at a community centre.

Measures

Functional mobility

A "get up and go" test was used to determine functional mobility before and after the TCC programme. A timed 6 m walk involved getting up from an arm chair, walking 3 m, turning back, walking back 3 m, and sitting down. This was used to test functional mobility related to balance and mobility.[22] Participants completed the timed test three days before the TCC programme and again three days after completion of the programme.

Health expectations for exercise

Outcome expectations for exercise (OEE) questionnaires were used to assess the physical and psychological health expectation of exercise before and after the TCC programme. The questionnaire includes nine items, each with a five point scale, developed to measure outcome expectations for exercise in adults by Resnick *et al*.[23] Permission to translate the OEE questionnaire into Chinese for this study was granted by Dr Resnick. It was first translated, then back translated to English, and verified by three bilingual researchers. The OEE questionnaire scales showed reliability, with the R^2 value ranging from 0.442 to 0.77, and validity, with path coefficients ranging from 0.69 to 0.87.[23] We recruited five experts to verify the content validity of the Chinese version of the questionnaire at 0.92. The internal consistency assessed in 12 participants revealed a Cronbach's α value of 0.80, and the test-retest reliability in 10 participants was 0.82. The questionnaire was completed before and after TCC for 12 weeks. Data collected were coded and double checked for statistical analysis. Changes in the OEE scores were used to reflect changed physical and psychological health expectations after the TCC exercise.

Complete blood counts and T lymphocyte subpopulations

Before and after the full 12 week programme, peripheral blood (3 ml) was collected in an anticoagulant EDTA tube. Two factors were analysed: the complete blood count and T lymphocyte subset. From the first 1 ml we measured complete blood count using an automatic haematology analyser (Fluidautomatic Cell Count, Sysmex Co, Long Grove, Illinois, USA) calibrated daily to ensure precision. An additional 1 ml was used to analyse the T lymphocyte CD4:CD8 ratio by direct immunofluorescent assay with fluorochrome conjugated monoclonal antibodies directed against CD3, CD4, and CD8 lymphocytes. The remaining 1 ml was used to analyse CD4CD25 regulatory T cells using dual staining with CD4 and CD25 monoclonal antibodies. Cell surface markers were analysed with a flow cytometer (FACSCalibur; Becton Dickinson, Palo Alto, California, USA), as previously described.[24]

Table 1 Comparisons of the physical and psychological expectations of exercise at the onset and after the end of a 12 week tai chi chuan exercise programme

Exercise expectation	Before	After	*t* Value	p Value
Makes me feel better physically	4.14 (0.54)	4.31 (0.67)	−1.139	0.263
Makes my mood better in general	4.06 (0.58)	4.25 (0.50)	−2.223	0.033
Helps me feel less tired	4.00 (0.53)	3.94 (0.83)	0.388	0.701
Makes my muscles stronger	3.92 (0.69)	4.06 (0.63)	−0.927	0.360
Is an activity I enjoy doing	3.89 (0.57)	4.39 (0.49)	−4.583	0.000
Gives me a sense of personal accomplishment	3.77 (0.81)	4.20 (0.76)	−2.674	0.011
Makes me more alert mentally	3.75 (0.77)	4.08 (0.73)	−2.415	0.021
Improves my endurance in performing my daily activities	4.00 (0.59)	4.14 (0.64)	−1.405	0.169
Helps to strengthen my bones	3.89 (0.75)	4.03 (0.65)	−1.044	0.304
Total scores	35.17 (4.10)	37.23 (4.44)	−2.616	0.013

Values presented are mean (SD) (n = 37). p Values were analysed by paired *t* test.
Outcome expectations for exercise included nine items, each with a five point scale from the lowest expectation (1) to the highest (5).

Table 2 Changes in complete blood count after the tai chi chuan exercise programme

Complete blood count	Before	After	p Value
Haemoglobin (g/l)	136 (2)	137 (3)	0.385
RBC ($\times 10^{-3}$ cells/mm^3)	4581.0 (77.6)	4646.2 (70.0)	0.213
WBC (cells/mm^3)	5715 (258.2)	5480.0 (275.7)	0.240
Neutrophils (cells/mm^3)	3116.7 (193.6)	3575.7 (304.2)	0.158
Monocytes (cells/mm^3)	320.0 (20.3)	229.8 (12.0)	<0.001
Lymphocytes (cells/mm^3)	1913.0 (83.7)	1969.5 (91.4)	0.557
Eosinophils (cells/mm^3)	124.9 (18.9)	130.7 (16.0)	0.702
Basophils (cells/mm^3)	27.0 (2.8)	28.1 (2.6)	0.742
Platelets ($\times 10^{-3}$ cells/mm^3)	209 (90)	223 (95)	0.166

Values presented are mean (SE) (n = 37). p Values were analysed by paired *t* test.
Complete blood counts were performed with an automatic haemocytometer which was calibrated daily to ensure precision.
RBC, Red blood count; WBC, white blood count.

Regulatory T cell mediators: transforming growth factor (TGF) β and IL10

Heparinised peripheral blood (5 ml) was collected from each participant before and after the TCC exercise programme. Peripheral blood leucocytes were harvested by dextran (4.5%) sedimentation. Mononuclear leucocytes were further isolated by gradient centrifugation in Ficoll-paque (Pharmacia Fine Chemicals, Piscataway, New Jersey, USA) at 1500 *g* for 20 minutes as previously described.[24] The mononuclear leucocytes (2×10^6 cells/ml) were suspended in a RPMI 1640 medium and challenged for one day with a varicella zoster virus antigen (vaccine strain; GlaxoSmithKline (GSK) Biochemicals, Rixensart, Belgium) at $10^{3.3}$ plaque forming units. After incubation for 24 hours, the reaction supernatants were harvested. Using an enzyme linked immunosorbent assay (ELISA), we measured TGFβ and IL10 concentrations, as previously described.[25]

Statistical analysis

Data were coded, reconfirmed, and analysed using descriptive analysis and paired *t* test. Version 12.0 of the Statistical Package for Social Sciences (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis. A significance level of 0.05 was chosen for statistical comparisons.

RESULTS

Changes in functional mobility

In the ''get up and go'' test of functional mobility performed before the exercise programme, the mean (SE) time for participants to complete the 6 m walking circuit was 6.33 (0.26) seconds. A highly significant (p = 0.001) decrease to 5.48 (0.22) seconds was found three days after the exercise programme was completed (fig 1).

Changes in health expectations

The participant's beliefs about the effect of exercise on physical and psychological health were assessed from the OEE questionnaire administered before and after completion of the TCC exercise programme. As shown in table 1, the results before exercise show higher physical than psychological health expectations. After the exercise programme, not only did the overall OEE significantly increase (p = 0.013), but there was also a notable increase in items linked with psychological health. Expectations that exercise improves mood, is enjoyable, gives a sense of accomplishment, and boosts mental alertness were significantly increased (table 1).

Changes in complete blood count

Complete blood counts were performed before and three days after the 12 weeks of TCC exercise. It was found that total white and red blood cells had not significantly changed after 12 weeks of TCC (table 2). Platelets and differential white blood cells, except monocytes, were not significantly different after TCC. Monocytes were significantly decreased (p<0.001).

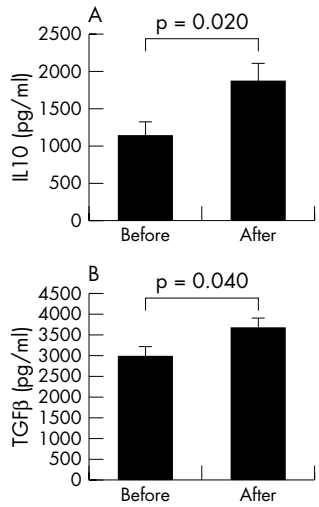

Figure 2 Mononuclear cells (2×10^6/ml) obtained from subjects before and after the 12 week tai chi chuan (TCC) exercise programme were challenged with varicella zoster virus antigen for one day. The culture supernatants were harvested for enzyme linked immunosorbent assay analysis of (A) interleukin 10 (IL10) and (B) transforming growth factor β (TGFβ) production. Both were significantly increased after the TCC exercise programme.

Table 3 Changes in lymphocyte subpopulations after the tai chi chuan exercise programme

Lymphocyte subpopulation	Before	After	p Value
CD4 (cells/mm^3)	831.5 (59.9)	842.5 (52.4)	0.819
CD8 (cells/mm^3)	588.6 (41.5)	582.0 (48.2)	0.937
CD4:CD8 ratio	1.5 (0.1)	1.6 (0.1)	0.050
CD4CD25 (cells/mm^3)	133.9 (12.5)	170.3 (13.6)	0.015

Values presented are mean (SE) (n = 37). p Values were analysed by paired *t* test.
Lymphocyte subpopulations were assessed by flow cytometry with dual staining techniques.

Changes in lymphocyte subpopulations

There was no significant difference in either CD4 lymphocytes or CD8 lymphocytes at the end of the TCC programme, but the CD4:CD8 ratio was significantly increased ($p = 0.05$; table 3). Moreover, CD4CD25 positive regulatory T cells had significantly increased by the end of the programme ($p = 0.015$).

Changes in regulatory T cell mediators: TGFβ and IL10

In addition to determining CD4CD25 regulatory T cells before and after TCC exercise, we also investigated whether the production of the regulatory cytokines TGFβ and IL10 was increased after TCC exercise. The production of TGFβ and IL10 by mononuclear leucocytes after varicella zoster virus stimulation was significantly increased at the end of the exercise programme (fig 2).

DISCUSSION

Many studies on exercise immunology were performed after a single session of exercise to exhaustion and show immunosuppression associated with risk of upper respiratory tract infections.[2–4] In this study, a regular programme of TCC lasting 60 minutes three times a week for 12 weeks significantly enhanced functional mobility and increased regulatory T cells and the regulatory mediators TGFβ and IL10. This research is the first to show that regular optimal exercise can increase regulatory T cells and augment regulatory T cell mediators in middle aged adults. Further studies to optimise the intensity and duration of TCC for better functional mobility and immunity regulation are needed.

Many studies have looked at possible ways to increase health benefits and improve immune function by an intervention of regular physical exercise. These interventions have consistently improved health in areas such as reduction of low density lipoproteins, increase in high density lipoprotein, improvement in balance and muscle strength, reduction of the tendency to fall, and increased self esteem.[5 10 14 26] Until now it has been controversial whether a regular moderate exercise programme could promote immune function. Schuler *et al*[27] showed that regular moderate exercise did not enhance specific antibody titres in young adults. A six month supervised exercise training programme led to nominal increases in some measures of immune function in previously sedentary elderly.[28] Another study with a progressive, moderate intensity exercise programme did not adversely affect the immune system but had a beneficial effect on the IL2/natural killer cell system.[29] Woods *et al*[30] showed that there was a tendency for an increase in CD4 and CD8 naive cells (CD45RA) and a decrease in CD4 memory cells (CD45RO) after the exercise intervention.

It is not clear how moderate exercise increases certain immune parameters. It is possible that immune enhancement due to extended moderate exercise comes indirectly from positive health benefits. Our study shows an increase in regulatory T cells associated with higher TGFβ and IL10 production under specific antigen (varicella zoster virus) stimulation, supporting this postulation. In support of the exercise modulation of regulatory T cell functions, Akimoto *et al*[31] also reported that 12 months of regular exercise training significantly increased salivary secretory I_gA concentrations in the elderly. Mucosal I_gA response is known to be critically regulated by TGFβ induction,[18 19] and TGFβ induction is also a critical step in autoimmune inflammatory disease.[20] Now we see that TCC may increase TGFβ production. This suggests that a TCC programme may improve mucosal defence leading to a lower risk of autoimmune and inflammatory disorders in adults. It is not clear why regular TCC for 12 weeks decreased monocytes in this study. This warrants further investigation.

TCC is known to promote health benefits by improving balance and muscle strength[5 14 32] and enhancing varicella zoster virus specific lymphocytes in older adults.[17] We show here that TCC exercise also promotes regulatory T cells and its mediators, improves balance, increases physical mobility, and enhances the perceived benefits of health in middle aged adults. These studies highlight that regular exercise benefits not only health but also immunoregulation.

What is already known on this topic

- Tai chi chuan (TCC) can improve flexibility and muscle strength
- Regular TCC exercise for 15 weeks increased varicella zoster virus specific lymphocytes in the elderly

What this study adds

- Regular TCC exercise for 12 weeks enhanced functional mobility, personal health expectations, and CD4CD25 regulatory T cells
- Production of the regulatory T cell mediators TGFβ and IL10 under varicella zoster virus stimulation was also significantly increased after TCC exercise

CONCLUSIONS

A 12 week regular TCC programme significantly improved functional mobility and attitudes about the outcomes of exercise. The T helper to suppressor cell ratio (CD4:CD8) showed a marginal increase, and regulatory T cells (CD4CD25) and their mediators TGFβ and IL10 under varicella zoster virus stimulation had also significantly increased after the TCC programme. In summary, regular TCC exercise for 12 weeks enhances functional mobility, health expectation, and regulatory T cell function.

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Competing interests: none declared

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